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New serological markers in medical mycology: (1,3)-β-D-glucan and *Aspergillus* galactomannan

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Abstract

Invasive fungal infections present a great challenge in modern medicine. The recent development of serologic markers represents a clear advance in the field. Two serological tests have been developed: (1,3)-β-D glucan and *Aspergillus* galactomannan. This review discusses highlights of both tests.

PALABRAS CLAVE
Infecciones fúngicas;
galactomanano de *Aspergillus;*
Pruebas serológicas

Nuevos marcadores serológicos en micología médica: (1,3)-βD-glucanos y galactomanano de *Aspergillus*

Resumen

Las infecciones invasoras por hongos representan un gran reto para la medicina moderna. El reciente desarrollo de marcadores serológicos representa un claro avance en este campo. Se han desarrollados dos pruebas serológicas: (1,3) - β-d-glucano y galactomanano de *Aspergillus*. Esta revisión recoge los aspectos más destacados de cada una de las pruebas.

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Introduction

Invasive fungal infections (IFI) present a great challenge in modern medicine. Due to the expansion of the population of immunosuppressed patients worldwide, the incidence of IFI has greatly increased, with high morbidity and mortality. One of the key elements for improving the outcome of IFI is the early initiation of appropriate antifungal treatment, which can be accomplished by the early diagnosis such infections. The problem is that the diagnosis of IFI is difficult, with non-specific and subtle clinical manifestations, and poor sensitivity of culture-based diagnostic tools[1].

The recent development of serological markers represents a clear advance in the field. While cryptococcal antigen is the standard of care for the diagnosis and management of cryptococcosis, until recently there were no serological tests to help in the diagnosis of important IFIs, such as invasive aspergillosis and candidiasis. Two serological tests have been developed: (1,3)-ß-D glucan (BG) and *Aspergillus* galactomannan. This review will discuss highlights of both tests.

(1,3)-ß-D-glucan

(1,3)-ß-D glucan is an important structural component of the majority of fungal cell walls[2]. Fungi that are known to have higher concentrations of BG in their cell walls include *Candida, Saccharomyces, Trichosporon, Sporothrix, Penicillium, Fusarium*, and *Aspergillus*. In general terms, molds such as *Scedosporium* and the agents of mucormycosis tend to have lower concentrations. Of note, *Cryptoccocus neoformans* has relatively low levels of this substance, and data are sparse for agents of the endemic mycoses, such as *Histoplasma, Coccidioides*, and *Blastomyces*[3]. As an almost pan-fungal marker, researchers have developed assays that are able to detect this complex carbohydrate in serum and variety of other fluids, such as bronchio-alveolar lavage fluid, cerebrospinal fluid, and abscess fluid[4,5].

The majority of assays are constructed as enzymatic/colorimetric methods based on the fact that BG triggers the coagulation cascade of the amebocyte cells of the horseshoe crab (*Tachypleus* in Asia and *Limulus* in North America) through the factor G pathway. It is also important to consider that amebocyte lysates from the different crab species have different affinity to BG, resulting in different cut-off values for different assays. Therefore it is very important to consider the specific kit and region of the world where it is made when interpreting results, as well as the related literature[6]. Other assays not relying on coagulation have been described, but have not undergone robust or large-scale validation.

It is widely considered that BG results represent active infection and are not affected by fungal colonization[7]. Antifungal therapy has not shown to interfere with the diagnostic performance of the assay either[8]. BG may be present in the bloodstream from a variety of other sources, such as surgical gauze, blood products, and filtered intravenous medications. It is important to consider such factors when interpreting results.

(1,3)-ß-glucan D as a panfungal diagnostic marker

Much of the initial validation of BG as diagnostic marker was carried out in Japan, where early studies reported high sensitivity and specificity in hospitalized patients with fever and fungal infection[9]. The first multicenter evaluation in North America was published in 2005, reporting that the sensitivity and specificity of the assay were 69.9% and 87.1%, respectively[8]. This study also showed that results were not affected by antifungal therapy. More recently, Karageorgopoulos et al.[6] performed a meta-analysis on 2979 patients from 16 large scale cohort and case-control studies assessing the diagnostic performance of BG, and reported a pooled sensitivity of 76.8% and specificity of 85.3%. Sensitivity was similar both for *Candida* and *Aspergillus*. BG is now considered alternative microbiological evidence of fungal infection in the latest version of the European Organization for Treatment and Research of Cancer/Mycosis Study Group criteria for diagnosis of fungal infection[10].

(1,3)-ß-D glucan as an outcome or prognosis assessment tool

Beyond diagnosis, where the Karageorgopoulos study established the final performance characteristics of the BG assay, attention has now been focused on the correlation of BG levels with outcomes and prognosis. Koo et al.[11] showed that BG tends to decline in patients with invasive candidiasis, aspergillosis and pneumocystosis, but failed to show prognostic value. In a cohort of patients with invasive candidiasis with an heterogeneous antifungal therapy background, Sims et al.[12] showed again that BG levels tend to decrease in successfully treated patients and increase in treatment failures. More recently, Jaijakul et al.[13] showed that in a large cohort of patients with invasive candidiasis treated with an echinocandin followed by an azole, the slope of a curve constructed with BG values correlated well with the treatment outcome. Furthermore, in that study a cut-off of <416pg/ml predicted a positive outcome with a positive predictive value of 89%.

Although evidence is accumulating for the use of BG for these purposes, this approach is still considered experimental and has not been endorsed by any guidelines.

Incorporating (1,3)-ß-D glucan into new management strategies for invasive fungal infections

Aside from the obvious diagnostic and prognostic applications of BG, and considering the limitations of current diagnostic techniques and the evidence that delayed diagnosis and treatment increase mortality, research is now focusing on incorporating BG monitoring as part of an early or "pre-emptive" treatment approach.

In a proof of concept study, Takesue et al.[14] followed a cohort of liver transplant recipients, following BG levels periodically and giving antifungal therapy when patients were febrile. In a retrospective analysis, they showed that patients who had positive BG results had a higher

likelihood of their fever responding to fluconazole. In a randomized pilot study, Hanson et al.[15] showed that patients randomized to receive anidulafungin triggered by positive BG results during twice weekly surveillance received antifungals more frequently than those who received it empirically or for a positive culture. The antifungal was well tolerated and there were numerically less proven and probable fungal infections, although the difference was not significant due to the sample size. More recently, Ostrosky-Zeichner et al. conducted a multicenter, randomized, placebo-controlled trial of caspofungin prophylaxis in high risk ICU patients (clinicaltrials.gov #NCT00520234). The study incorporated BG measurements twice weekly as a way to identify prophylaxis failures early and allowed for caspofungin to be used in an open label fashion on those patients, becoming functionally a pre-emptive therapy study nested in the main prophylaxis trial. The authors showed that the BG-based pre-emptive therapy strategy decreased the incidence of proven and probable invasive candidiasis cases.

Although exciting advances have been done in this area, further research is required to assess the efficacy of these types of interventions on major patient and economic outcomes.

Galactomannan

Invasive aspergillosis (IA) has become an important cause of death in patients under risk, such as those suffering from hematological malignancies, those undergoing hematopoietic cell transplantation, and solid organ transplant recipients (especially of the lung)[16]. Galactomannan (GM) is a polysaccharide released by growing *Aspergillus* hyphae that can be measured in the serum and other body fluids. The Platelia® *Aspergillus* EIA is a one stage immunoenzymatic sandwich microplate assay which detects circulating GM from body fluids (mainly serum and bronchoalveolar lavage). It uses rat EBA-2 monoclonal antibodies which are directed against *Aspergillus* galactomannan. The result is expressed as an index.

Galactomannan in the serum to early diagnose invasive aspergillosis

The serum galactomannan index assay (GMI) has been widely used for the diagnosis of IA, with an excellent performance in neutropenic patients with hematological malignancies or in the pre-engraftment phase post-hematopoietic cell transplantation. The sensitivity of the test in patients not receiving antifungal therapy was reported as 87.5%[17]. In another study, the sensitivity and specificity of the GM test was shown to be 96.8% and 82.4%, respectively[18]. The best use of GMI in this setting is as a screening method to early diagnose IA or to trigger the initiation of antifungal therapy (diagnostic-driven or preemptive antifungal therapy). A typical protocol for early diagnosis or screening is to perform the test three times per week. The easiest way of using GMI in this context is to rely on its high negative predictive value and rule out IA when the test is repeatedly negative. However, one must be alert to the fact that false-negative tests may occur when the patient is receiving an anti-mold antifungal agent[19]. Another important cause of false-negative test is if the patient is no longer neutropenic. Therefore, if the patient is neutropenic and is not receiving an anti-mold antifungal agent, repeatedly negative GMI is good at ruling out IA. On the other hand, once a patient develops one positive test (defined when the index value in serum is =>0.5) additional measures are made according to the pre-test probability of IA. In this context, the clinician should rely on a curve rather than on just a single test. Because animal models have consistently shown a sharp relationship between serum GMI and fungal burden in tissue[20], the best interpretation for an ascending GMI curve is that the patient is developing IA. In such circumstance, additional tests are ordered, including chest and sinuses computed tomography. Indeed, we described a form of early IA in patients with multiple myeloma in which patients had host factors, clinical features and mycological criteria for IA, but lacked the typical (and later) images of well-circumscribed lesions, air crescent or cavity on chest CT[21]. Instead, those patients had non-specific infiltrates (patchy, ground-glass, tree in bud) that eventually evolved to specific well-circumscribed lung infiltrates. A similar observation was made later by other authors in patients with acute myeloid leukemia, patients with lymphoproliferative diseases, and in allogeneic hematopoietic cell transplantation recipients with graft versus host disease[22].

A consequence of the early diagnosis of IA is that the outcome is improved because the disease is being treated with a low fungal burden in the same way that the outcome of neoplastic diseases is better the lower the tumor burden.

Galactomannan in the serum to monitor treatment of invasive aspergillosis

Treatment response in IA has relied on composite response criteria that take into account improvement in clinical findings and imaging. The problem is that symptoms are nonspecific and radiological images frequently worsen during the course of treatment of IA, including when neutrophils recover, creating an inflammatory immune reconstitution syndrome[23]. Specifically, currently accepted criteria define response with a specific percentage of reduction in the volume of infiltrates. However, the reliability and reproducibility of such readings are greatly imprecise. On the other hand, GMI trends down early in the course of disease, when patients are responding to treatment, and its kinetics may be very useful to predict the outcome. We recently compared the European Organization for Treatment and Research of Cancer/Mycosis Study Group response criteria with criteria based on the kinetics of GMI. We defined success as survival plus repeatedly negative serum GMI for ≥2 weeks after the first negative GMI in the absence of new extra-pulmonary IA lesions and failure as a persistently positive serum GMI. Death within the 14 day-period was considered as failure, unless autopsy examination failed to reveal IA. Among 115 patients with IA, agreement was observed in 91% of cases, with 100% agreement in failure and 87% in success[24]. More recently, we reported that normalization of GMI within 7 days from the first positive test was strongly associated with the outcome[25]. Therefore, a simple and objective serum biomarker may be useful to monitor therapy as early as seven days after diagnosis.

Galactomannan in the serum in non-neutropenic patients

While GMI is very useful in neutropenic patients (as discussed earlier), its value in non-neutropenic patients is less certain. In one study the sensitivity of GMI was 23.1% and the specificity was 76.1%, with a positive predictive value of 1.6% only, but a negative predictive value of 98.3%[26]. In non-neutropenic patients false-negative results are more frequent. Therefore, the best use of GMI is when results of sequential tests trend up. In this setting, every attempt to confirm the diagnosis of IA should be advanced.

False positive results

Several possible causes of false-positive reactions have been described, among which the direct interaction of some antibiotics with the Platelia *Aspergillus* test has been recently highlighted. Such cross-reactivities have been found mainly for some ß-lactams but not for other commonly used antibiotics of fungal origin (penicillins and cephalosporins), non-fungal origin (erythromycin, gentamicin, and vancomycin), and synthetic origin (quinolones). The clinical relevance of this cause of false-positive results has been recently challenged[27]. It is important to note that GM is a complex sugar also found in many food products. In addition, lipoteichoic acid from the bacterium *Bifidobacterium* may also cross-react with the assay, and false-positive results may occur owing to high bacterial loads of *Bifidobacterium* in the gut of pediatric patients[28]. In addition, other fungi may cross-react for the GMI test. These include *Fusarium, Histoplasma capsulatum, Alternaria, Penicillium* and *Paecylomyces*[29].

Galactomannan detection in bronchoalveolar lavage

Detection of GMI in the bronchoalveolar lavage (BAL) has become a very important tool for the diagnosis of IA. Several studies have been performed in order to evaluate the usefulness of this assay and the more appropriate cut-off index. In one study in 128 patients with hematological diseases and pulmonary infiltrates, GMI in the BAL performed better than conventional methods for the microbiological diagnosis of IA, both in neutropenic and non-neutropenic hematology patients[30]. The sensitivity of BAL GMI (at a cut-off of ≥1.0) was 91.3%, compared with 50% and 53% for culture and direct examination, respectively. In 20 patients GMI positivity was the only microbiological finding for IA on BAL samples. Conversely, negative BAL GMI ruled out IA with a very high negative predictive value (96%, at a cut-off of ≥1.0). A sensitivity of almost 97% was also observed in 31 proven IA with a cut-off for positivity of ≥1. This high sensitivity was consistent with other reports in hematology patients[31], in solid-organ transplant recipients[32], and non-immunocompromised patients (all using the ≥1 cut-off), and in critically ill neutropenic patients (using the≥ 0.5 cut-off)[33,34]. In a more recent study, the performance of GM in BAL in patients at risk was performed, and evaluated by using a range of index cut-offs to define positivity. Using a BAL fluid GM index of ≥0.8 the sensitivity in diagnosing proven and probable IA was 86.4%, and the specificity was 90.7%. At this cut-off, the positive and negative predictive values were 81% and 93.6% respectively. An OD index value ≥3.0 corresponded to 100% specificity, and an OD index cut-off of <0.5 corresponded to a high sensitivity, virtually always ruling the disease out. For all values in between, the probability of IPA depends largely on other factors[35].

In the case of IA in lung transplant recipients, it was observed that using an index value of 0.5 for the serum, GM detection in BAL had a sensitivity of 60% and a specificity of 95%. The specificity increased to 98% when the index cut-off value of 1 was used, while the sensitivity remained the same[36]. In another study the positive predictive value of a positive BAL GM test was low at the 0.5 cut-off (24.2%). However, the increase in the specificity improved without compromising sensitivity. The best cut-off was defined at 1.5 (sensitivity 100% and specificity 90.4%)[37].

Conclusions

Galactomannan assay is a useful tool for detection of IA in patients at high risk. The cut-off value in serum was ≥ 0.5, and in BAL the best cut-off was ≥ 1.5. Serum GMI helps to early diagnose and monitor IA therapy in neutropenic patients.

Transparency declarations

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